translocation step in the case of a transporter or a pore gating step in K2P channels.

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Can ClC-2 Chloride Channel be Activated by Hyperpolarization Alone in Cells Dialyzed with Non-Permeant Anions?

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Open and closing of ion channels is driven by conformational changes triggered by either an intrinsic voltage sensor in voltage-gated channels or by ligand binding in ligand-gated channels. However, ClC-2, a two pore Cl^- channel is activated by hyperpolarisations despite of lacking of an intrinsic voltage sensor. The structural information available to date strongly suggests that in the closed conformation each pore of ClC-2 is occluded by the negatively charged side chain of a glutamate residue. Although the gating mechanism is unknown, there is evidence which indicates that intracellular anions and extracellular protons regulate gating. The present work was aimed to determine the contribution of pore occupancy caused by intracellular anions in gating the mouse ClC-2 channel. In our experiments, ClC-2 was readily activated by hyperpolarisations when permeant anions such Cl^-, SCN^-, Br^- and I^- were present on either side of the membrane. However, ClC-2 was not activated in cells dialyzed with acetate (0 Cl^-) and exposed to $[H^+]_O=10^{-7.3}$ M. In contrast, the channels were opened by increasing $[Cl^-]_i$ at $[H^+]_O=10^{-10}$ M_a condition unlikely to protonate the glutamate's side chain. Importantly, voltage gating occurred when F^- or glutamate were present in the cytosolic side. Since these two anions are non-permeant we propose that they must enter the pore and interact with the glutamate side chain from the inside in order to induce opening. Thus, we propose that a strong hyperpolarisation drives the intracellular anions into the permeation pathway and the subsequent electrostatic interaction between the anions and the negatively charged side chain opens the pore. Our data support the hypothesis that voltage-dependent gating in mouse ClC-2 requires intracellular anions. Supported by grant 219949 from CONACyT.

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Molecular Basis of Voltage-Dependent Gating in ClC Transporters

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The CLC family encompasses Cl^- channels and coupled Cl^-/H^+ exchangers. CLC channels and transporters both exhibit voltage-dependent gating, the physiological importance of which is illustrated by multiple disease-causing mutations that specifically result in altered voltage sensing. Despite a large body of available functional and structural data, the molecular mechanisms underlying voltage gating in CLCs still remain unclear. Using electrophysiological admittance measurements, we recently decomposed voltage-dependent activation of the human ClC-5 transporter into multiple discrete electrogenic transitions (Grieschat M & Alekov AK (2014) *Biophys J* 107, L13–L15). A key player of the gating machinery appears to be the so-called gating glutamate (Glu_{ext}; Glu148 in EcClC), which is thought to move upon changes in voltage. To better understand the basis of voltage-dependent gating, we conducted molecular dynamics simulations of the bacterial homologue EcClC. Using a double-bilayer system, we applied small ionic concentration gradients across the membrane that resulted in charge imbalances Δq_{ion} and we calculated the resulting potential differences V_m. Analysis of the dependence of V_m on Δq_{ion} for various conformations that differed in Cl^- binding site occupancy and the protonation state of Glu_{ext} was used to determine charge displacements of the transporter along the electric field. Multiple processes, including protonation and conformational changes of Glu_{ext}, binding of Cl^- ions and refocusing of the electric field triggered by changes in water accessibility are associated with charge transfer across the membrane and therefore exhibit intrinsic voltage dependence. We calculated gating charges that underlie these conformational transitions of the CLC transporter. Based on these calculations, gating charge recordings and capacitance measurements on ClC-5, we propose a molecular description of CLC voltage sensing which attributes fractional gating charges to these partial reactions of the transport cycle.

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Multiphasic Profiles: Discontinuous Transitions in Conductance-Voltage Data for Ion Channels

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Multiphasic profiles, a series of straight lines separated by discontinuous transitions, were first found (Nissen, Annu. Rev. Plant Physiol. 1974) for the concentration-dependence, plotted in linear transformations of the Michaelis-Menten equation, for ion uptake in plants. Reanalyses of recently published data show that conductance-voltage data for ion channels are also well represented as multiphasic. In contrast, the Boltzmann function and other functions giving curvilinear profiles must often be rejected for statistical reasons (very low probabilities by the Runs test that the uneven distribution of points around the profiles is due to chance). Plots of deviates show that the fits to multiphasic profiles are usually better than the fits to curvilinear profiles, i.e. that the data are more precise than apparent from the published plots. As shown by simulation, the better fits to multiphasic profiles are not due to errors (noise) in the data. Adjacent lines are often parallel. The transitions are then necessarily in the form of noncontiguities (jumps). Non-parallel lines are also frequently separated by jumps. Most often, replicate determinations give different multiphasic patterns (number of phases, voltages at which the transitions occur). The use of the averages will then give a meaningless pattern.

Whenever the data are sufficiently detailed and precise, multiphasic profiles are also found for a variety of other processes and systems, biological as well as nonbiological: 1) pH profiles, including profiles for non-enzymatic model systems. 2) Binding as determined by fluorescence anisotropy titration or isothermal titration calorimetry (ITC). 3) Folding and unfolding of proteins. Implications for the opening and closing of single biological channels will be discussed.

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A Highly Cooperative and Steeply Voltage Gated Channel Triplet

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When reconstituted into planar phospholipid membranes, a 4.5nS (in 1M KCl) membrane channel complex behaves as if it is composed of three channels that are operating in a highly cooperative manner. The channels are steeply voltage-gated (e-fold for 1.8 mV) and well-organized. In the ± 70mV range, no voltage gating takes place until the first channel, channel 1, closes at high positive potentials (>70 mV). Only following this closure does channel 2 begin to gate in response changes in voltage. For channel 2, closure takes place at low negative potentials (typically −25 to −30 mV). It is only when channel 2 is closed that channel 3 closes routinely but does so at low positive potentials (typically 25-30 mV). Simultaneous reopening of channels 2 and 3 is common, demonstrating especially high cooperativity. These and other remarkable properties support a working model for the gating and cooperativity of these channels. The highlights include anti-parallel orientation of the channels and the voltage sensor dipole being responsible both for voltage gating and cooperativity. (Supported by NSF grant MCB-1023008)